



King's Research Portal

DOI:

[10.4161/rdis.27109](https://doi.org/10.4161/rdis.27109)

Document Version

Publisher's PDF, also known as Version of record

[Link to publication record in King's Research Portal](#)

Citation for published version (APA):

Yannakoudakis, B., & Liu, K. (2013). Common skeletal features in rare diseases: New links between ciliopathies and FGF-related syndromes. *Rare Diseases*, 1(1), [e27109]. <https://doi.org/10.4161/rdis.27109>

Citing this paper

Please note that where the full-text provided on King's Research Portal is the Author Accepted Manuscript or Post-Print version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version for pagination, volume/issue, and date of publication details. And where the final published version is provided on the Research Portal, if citing you are again advised to check the publisher's website for any subsequent corrections.

General rights

Copyright and moral rights for the publications made accessible in the Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the Research Portal

Take down policy

If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Common skeletal features in rare diseases

New links between ciliopathies and FGF-related syndromes

Basil Z Yannakoudakis and Karen J Liu*

Department of Craniofacial Development and Stem Cell Biology; King's College London; London, UK

Keywords: ciliopathy, FGF, skeletal dysplasia

Abbreviations: C, cleft; CF, cervical fusion; CS, coronal synostosis; HA, high arch; PD, polydactyly; SD, syndactyly; SL, short limbs; SS, sagittal synostosis

*Correspondence to: Karen J Liu;
Email: karen.liu@kcl.ac.uk

Submitted: 08/18/2013; Revised: 10/24/2013;

Accepted: 11/06/2013;

Published Online: 12/03/2013

<http://dx.doi.org/10.4161/rdis.27109>

Citation: Yannakoudakis BZ, Liu KJ. Common skeletal features in rare diseases: New links between ciliopathies and FGF-related syndromes. *Rare Diseases* 2013; 1:e27109

Addendum to: Tabler JM, Barrell WB, Szabo-Rogers HL, Healy C, Yeung Y, Perdiguero EG, Schulz C, Yannakoudakis BZ, Mesbahi A, Wlodarczyk B, et al. Fuz mutant mice reveal shared mechanisms between ciliopathies and FGF-related syndromes. *Dev Cell* 2013; 25:623-35; PMID:23806618; <http://dx.doi.org/10.1016/j.devcel.2013.05.021>

Congenital skeletal anomalies are rare disorders, with a subset affecting both the cranial and appendicular skeleton. Two categories, craniosynostosis syndromes and chondrodysplasias, frequently result from aberrant regulation of the fibroblast growth factor (FGF) signaling pathway. Our recent work has implicated FGF signaling in a third category: ciliopathic skeletal dysplasias. In this work, we have used mouse mutants in two ciliopathy genes, *Fuzzy* (*Fuz*) and *orofacial digital syndrome-1* (*Ofd-1*), to demonstrate increase in *Fgf8* gene expression during critical stages of embryogenesis. While the mechanisms underlying FGF dysregulation differ in the different syndromes, our data raise the possibility that convergence on FGF signal transduction may underlie a wide range of skeletal anomalies. Here, we provide additional evidence of the skeletal phenotypes from the *Fuz* mouse model and highlight similarities between human ciliopathies and FGF-related syndromes.

Fibroblast growth factors are well-studied signaling molecules that are critical for embryonic development.^{1,2} In humans, 22 structurally related FGF ligands have been identified; most of these are secreted proteins that bind with varying affinities to tyrosine kinase receptors (FGFR1–4). The majority of FGF ligands can bind promiscuously to multiple different FGFRs; further complexity is generated by alternative splicing of FGFR1, 2 and 3. Ligand binding induces receptor dimerization and cross-phosphorylation, which then initiates a broad range of

intracellular signaling cascades such as PI3 kinase (PI3K), MAPK, phospholipase C (PLC γ) or JAK/STAT. Activation of the pathway elicits diverse cellular responses, including proliferation and differentiation of multiple cell types. In recent years, developmental studies, as well as identification of human alleles, has made it clear that precise temporal and spatial activation of FGF signaling is necessary for normal development.

The FGF signaling pathway is a key regulator of skeletal development. In humans, mutations in the FGF receptors (FGFRs) are a hallmark of two classes of skeletal anomalies: craniosynostoses and chondrodysplasias (reviewed by Ornitz et al.).¹ Craniosynostosis syndromes are characterized by premature fusion of the cranial sutures and are frequently accompanied by malformations of the axial skeleton. In contrast, chondrodysplasias feature truncation of the appendicular skeleton, due to an increase in FGF signaling during endochondral ossification. Both craniosynostosis and chondrodysplasia syndromes are associated with autosomal dominant mutations of the FGF receptors, which result in increased activation of the signaling pathway.¹ The array of phenotypes correlates with specific mutations in different receptors, but the net effect appears to be that sustained or increased FGF signaling tips the balance between critical steps in osteoblast differentiation.^{1,2}

Ciliopathies are a heterogeneous group of disorders that arise from abnormal formation or function of the cilium.³ Cilia are finger-like organelles at the cell surface

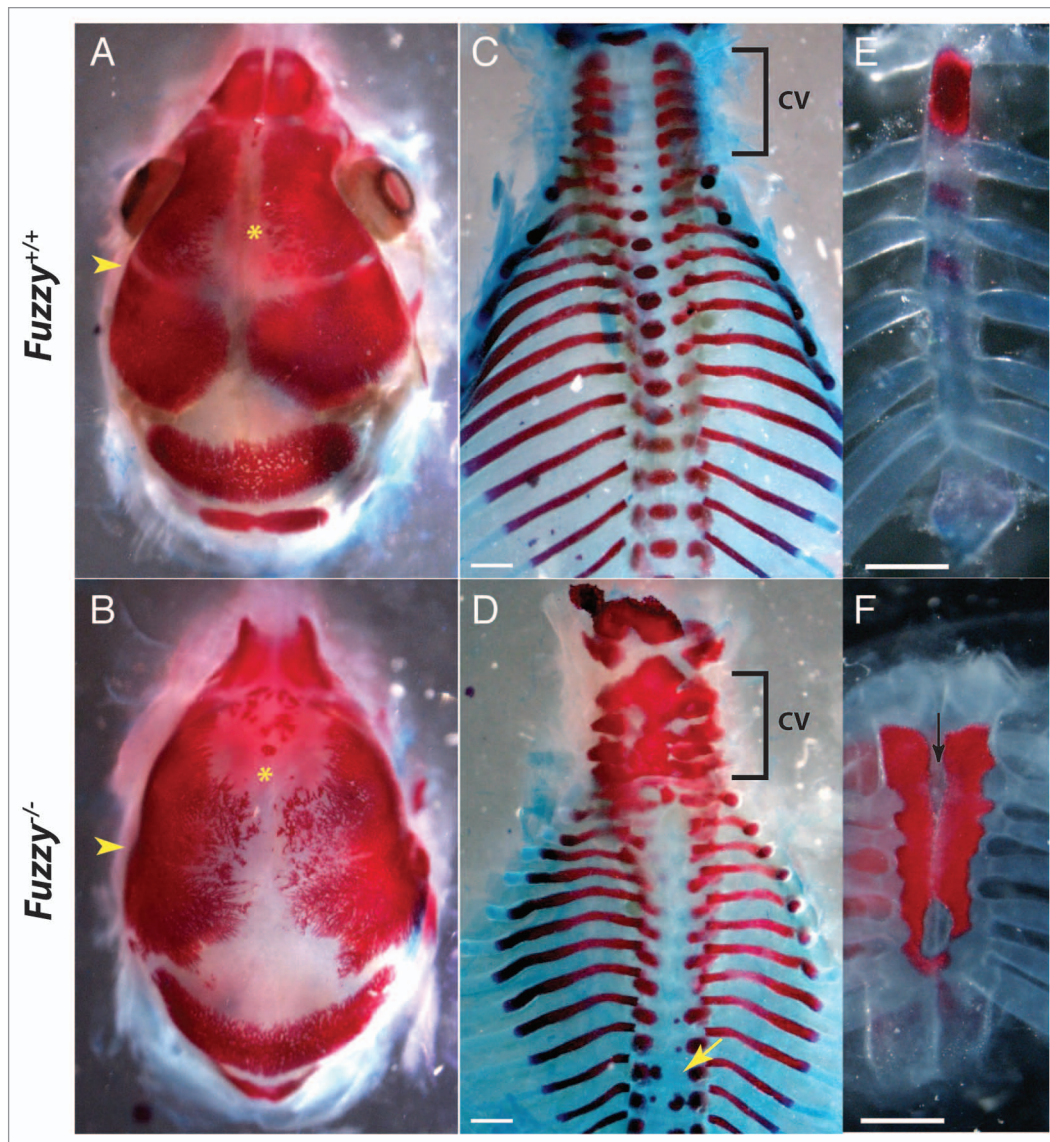


Figure 1. Skeletal preparations of wild-type and *Fuz*^{-/-} embryos at E18.5. Alizarin red staining marks the bone. Alcian blue staining marks the cartilage. **(A and B)** Dorsal views of the skull. **(A)** Control. **(B)** Mutant mice display synostosis of the coronal suture (yellow arrowhead) as well as an open anterior fontanelle (yellow asterisk). **(C and D)** Dorsal view of the axial skeleton. **(C)** Control. **(D)** In mutant animals, the cervical vertebra (cv, bracket) are fused. Ossification of the centrum in thoracic vertebra is lost or aberrant (yellow arrow). **(E and F)** Frontal views of the sternum. **(E)** Control. **(F)** In mutants, the sternum is shorter, hyperossified and cleft/bifid (black arrow).

comprised of a microtubule axoneme attached to a basal body. Depending on the microtubule arrangement, a cilium may be motile or immotile. Disorders of the motile cilia frequently involve fluid flow; for example, patients with primary ciliary dyskinesia have difficulty clearing mucus from their lungs due to defects in the multi-ciliated epithelium.⁴ Immotile or primary cilia stand alone and are required for function of many signaling pathways.⁵ To date, ciliopathic skeletal phenotypes have mainly been attributed to changes

in primary cilia-dependent transduction of Hedgehog signals.⁶ This has been best studied in the long bones; however, the phenotypic range of ciliopathies is extremely broad. As a consequence, it can be difficult to diagnose or treat ciliopathies, and the underlying etiology is often unclear.

Ciliopathies affecting the skeleton are rare syndromic anomalies. Some patients exhibit limb phenotypes such as syndactyly or polydactyly [in the case of Bardet-Biedl syndrome (MIM

#209900)].^{7,8} Dysplasia of the ribs, and occasional shortened limbs, are also seen, as in Jeune asphyxiating thoracic dystrophy (MIM #208500).⁹ Frequently, ciliary defects also lead to changes in the craniofacial skeleton in humans and mice,¹⁰⁻¹² with craniosynostosis observed in Sensenbrenner syndrome or cranioectodermal dysplasia (MIM #614378).¹³ The broad range of systems affected suggests varied molecular causes. Thus, grouping similar skeletal phenotypes across multiple disorders, and

Table 1. Skeletal phenotypes observed in ciliopathies and FGF syndromes. Included are selected human disorders and animal models. Unfortunately, due to space constraints, we regret that we are unable to cite all relevant papers.

	Affected structure						
	Skull/Face	Palate	Limb/Hand	Vertebra	Rib/Thorax	Sternum	Refs.
Ciliopathies							
Human							
Meckel	encephalocele	C	PD	?	-	-	20
Bardet-Biedl	Shape change	HA	PD, SD	scoliosis	-	-	37
Joubert	-	C?	-	Cervical fusion	-	-	28
OFD1	Shape change	HA	PD	-	-	-	20
Jeune	-	-	PD	Cervical stenosis	irregular	bulge	9, 38
Sensenbrenner	SS	HA	SL, PD	-	Short ribs	-	13, 39–41
Ellis-Van Creveld	-	-	SL, PD	-	Short ribs	-	42
Mouse Models							
Meckel: <i>MKS1^{h1b614}/MKS1^{krc}</i>	-	C	-	-	-	bifid, fused	35, 43
Ellis-van Creveld: <i>EVC</i>	-	-	SL	Fusions	Short ribs	-	44, 45
Orofacial Digital: <i>OFD1</i>	-	C	SL, PD	-	-	bifid, fused	46, 47
Sensenbrenner: <i>WDR35</i>	-	-	-	-	Short ribs	-	48
Ciliopathy: <i>Fuz</i>	CS	HA	SL, PD	Cervical fusion	Short ribs	Fused, bifid	14, 16–18
Craniosynostosis							
Human							
Apert	CS	HA	SD	Cervical fusion	-	-	19, 49
Crouzon	CS	HA	SL, SD	Cervical fusion	-	-	21, 50
Pfeiffer	CS	HA	SD	Cervical fusion	-	-	22, 23
Mouse Models							
Crouzon/Pfeiffer: <i>Fgfr2c^{C342Y}</i>	CS	C	-	-	-	Fused	51, 52
Pfeiffer: Tg(<i>Fgfr1^{P252R}</i>)	CS	-	PD	Homeotic transformation	-	Fusions	27
Apert: <i>Fgfr2^{IIIc/Δ}</i>	CS	-	-	-	-	Fused	29, 53
Apert: <i>Fgfr2^{S252W}</i>	CS	C	-	-	-	Fusions	30
Chondrodysplasia							
Human							
Chondrodysplasia punctata 2	variable	-	SL	Scoliosis fusions	calcified	calcified	20
Hypochondroplasia	variable	-	SL	stenosis	-	-	53
Thanatophoric dysplasia	-	-	SL	flattened	Short ribs	-	54
Achondroplasia	Small base	-	SL	stenosis	Small chest	-	55
Mouse Models							
Achondroplasia: <i>Fgfr3^{G374R}</i>	-	-	SL	Cervical fusion	-	-	56
Thanatophoric Dysplasia: FGFR3369	-	-	SL	-	Short ribs	-	57

Abbreviations: SS, sagittal synostosis; CS, coronal synostosis; C, cleft palate; HA, high arched palate; PD, polydactyly; SD, syndactyly; SL, short limbs

comparison with animal models, may provide useful insight into the underlying molecular events.

The *Fuzzy* gene is associated with neural tube defects and has previously been shown to be a ciliopathy gene.^{14–17} In our current

work, we examine the requirements for *Fuz* in development of the craniofacial structures.¹⁸ Craniofacial defects include craniosynostosis and facial anomalies (Fig. 1A and B).¹⁸ As documented in Tabler et al., *Fuz* mutant mice have a

complete synostosis of the coronal suture, as well as an open anterior fontanelle, reminiscent of Apert syndrome synostoses (MIM #101200) (Fig. 1A-B).¹⁹ Our analysis of the *Fuzzy* mutant also showed broader defects of the skeleton. Consistent

with ciliopathic Hedgehog phenotypes, *Fuz* mutants have polydactyly and shortened long bones.¹⁷ Most interesting, we also found anomalous elements in the axial skeleton. The cervical vertebra (cv) were frequently fused (Fig. 1C and D, bracket), while ossification of the centrum in the thoracic vertebra is generally absent. Occasionally, small islands of ectopic ossification are seen (Fig. 1C and D, yellow arrow). As in ciliopathies such as Jeune Syndrome (MIM #208500), the ribs are shorter. Surprisingly, the sternum is hyperossified, shorter, and bifid (Fig. 1E and F, black arrow marks cleft). In earlier stages, cartilaginous joints are formed (data not shown); by embryonic day (E)18.5, the sternal joints have been obliterated (Fig. 1E and F).

This array of phenotypes suggested a similarity to several classes of FGFR-dependent skeletal anomalies, including craniosynostosis syndromes and chondrodysplasias.²⁰ All of these syndromes arise from dysregulation of FGF receptors;¹ however, the status of FGF signaling in ciliopathy mutants has not been well explored. Table 1 catalogs the skeletal malformations ciliopathies and FGF related syndromes. We found significant overlap across the range of disorders.

Many craniosynostosis patients have a progressive fusion of the cervical spine, with two thirds of Apert patients exhibiting complex fusions in the C5-C6 segment.²¹ C2-C3 fusions are also quite common in these syndromes, with additional reports from Saethre-Chotzen (MIM #101400) and Pfeiffer syndromes (MIM#101600).²²⁻²⁴ To our knowledge, FGF-induced chondrodysplasias are not associated with spinal stenosis; however, cases have been reported in rhizomelic chondrodysplasia punctata patients.²⁵ Interestingly, congenital scoliosis due to vertebral defects has also been linked to aberrant *FGF* signaling during development.²⁶ In addition, in a mouse model of Pfeiffer syndrome, vertebral homeotic transformations have been noted.²⁷ Thus, phenotypes seen in

the different mouse models, combined with some reported anomalies of the cervical vertebra in Joubert Syndrome (MIM #213300), suggest that ciliopathy patients could be assessed for spinal aberrations.²⁸

Sternal abnormalities, a common feature of ciliopathies and FGF syndromes, are another striking phenotype seen in *Fuz* mutants. Premature or ectopic fusion of the sternum is seen in a number of mouse models, including Apert/Pfeiffer Syndrome and achondroplasia mice.^{27,29-31} The current data suggest that hyperactivation of FGF receptors leads to an impairment in sternal joint formation and subsequent hyperossification. In humans, premature ossification of the sternum is a hallmark of Noonan syndrome.³² The causative mutation in Noonan syndrome is *PTPN11*, which encodes SHP-2, a key regulator of the FGF-Ras-MAPK pathway.³³ Finally, sternal anomalies are also observed in ciliopathic animal models.^{34,35}

Taken together, our data suggest that the skeletal anomalies described may all converge on deregulation of the FGF signaling pathway. Indeed, we found that a subset of phenotypes in our ciliopathic mouse mutants, *Fuz* and *OFD-1*, are attributable to increased *Fgf8* gene expression and genetic reduction of *FGF8* rescued these phenotypes.¹⁸

Our approach of cataloguing human phenotypes, and comparison to animal models, led us to a surprising role for FGFs in ciliopathies. However, clearly, FGFs alone, or in combination with Hedgehog signaling, cannot be the sole molecular players in skeletal dysplasias. FGF signaling plays roles at multiple steps in both endochondral and intramembranous ossification.¹ For example, during long bone formation, signaling via FGFR2 and FGFR3 promotes chondrocyte condensation and differentiation respectively. Later in this process, FGFR3 is needed to limit the amounts of proliferative pre-hypertrophic chondrocytes. In intramembranous ossification, as seen in

the calvaria, FGFs are involved in every step of osteoblast differentiation and subsequent ossification. *FGFR1* and 2 are expressed at the osteogenic front, and are necessary for osteoblast differentiation. *FGF2* is expressed in the sutures, and signals to the adjacent osteogenic cells. Thus, specific phenotypes observed in the human syndromes described can be attributed to inappropriate temporal or spatial activation of the pathway. For example, hyperactivation of FGFR3 severely reduces regions of pre-hypertrophic chondrocyte proliferation resulting in short long bones. Conversely, FGFR1 and 2 dysregulation leads to premature osteoblast differentiation and craniosynostosis.

Dysregulation of Gli processing is also known to cause a variety of skeletal defects, notably in the long bones, vertebra and sternum. *Gli2* mouse mutants have shortened long bones and absence or malformation of vertebral bodies, while *Gli3* mutants have slightly shortened long bones accompanied by polydactyly, as well as fusions of the cervical vertebra and bifid, hyperossified sterna.³⁶ Thus, there is substantial phenotypic overlap between *Gli* mutants and other animal models of skeletal syndromes. As described above, it is likely that correct timing and location of a suite of signals is critical for shaping the skeleton. Because pathological mutations can lead to changes at multiple levels during development, we propose that further comparison of human phenotypes and animal models can provide important insights into the genetic networks governing overlapping disease phenotypes.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

Acknowledgments

We thank the Liu lab for discussions and Marc Dionne for critical reading of the manuscript. This work was funded by grants from the Wellcome Trust, BBSRC, and an MRC studentship.

References

- Ornitz DM, Marie PJ. FGF signaling pathways in endochondral and intramembranous bone development and human genetic disease. *Genes Dev* 2002; 16:1446-65; PMID:12080084; <http://dx.doi.org/10.1101/gad.990702>
- Mohammadi M, Olsen SK, Ibrahim OA. Structural basis for fibroblast growth factor receptor activation. *Cytokine Growth Factor Rev* 2005; 16:107-37; PMID:15863029; <http://dx.doi.org/10.1016/j.cytogfr.2005.01.008>
- Veland IR, Awan A, Pedersen LB, Yoder BK, Christensen ST. Primary cilia and signaling pathways in mammalian development, health and disease. *Nephron Physiol* 2009; 111:39-53; PMID:19276629; <http://dx.doi.org/10.1159/000208212>
- Rossman CM, Forrest JB, Lee RM, Newhouse MT. The dyskinetic cilia syndrome. Ciliary motility in immotile cilia syndrome. *Chest* 1980; 78:580-2; PMID:6448132; <http://dx.doi.org/10.1378/chest.78.4.580>
- Eggenchwiler JT, Anderson KV. Cilia and developmental signaling. *Annu Rev Cell Dev Biol* 2007; 23:345-73; PMID:17506691; <http://dx.doi.org/10.1146/annurev.cellbio.23.090506.123249>
- Haycraft CJ, Serra R. Cilia involvement in patterning and maintenance of the skeleton. *Curr Top Dev Biol* 2008; 85:303-32; PMID:19147010; [http://dx.doi.org/10.1016/S0070-2153\(08\)00811-9](http://dx.doi.org/10.1016/S0070-2153(08)00811-9)
- David A, Bitoun P, Lacombe D, Lambert JC, Nivelon A, Vigneron J, Verloes A. Hydrometrocolpos and polydactyly: a common neonatal presentation of Bardet-Biedl and McKusick-Kaufman syndromes. *J Med Genet* 1999; 36:599-603; PMID:10465109
- Beales PL, Elcioglu N, Woolf AS, Parker D, Flinter FA. New criteria for improved diagnosis of Bardet-Biedl syndrome: results of a population survey. *J Med Genet* 1999; 36:437-46; PMID:10874630
- Baujat G, Huber C, El Hokayem J, Caumes R, Do Ngoc Thanh C, David A, Delezoide AL, Dieux-Coelster A, Estournet B, Francannet C, et al. Asphyxiating thoracic dysplasia: clinical and molecular review of 39 families. *J Med Genet* 2013; 50:91-8; PMID:23339108; <http://dx.doi.org/10.1136/jmedgenet-2012-101282>
- Brugmann SA, Cordero DR, Helms JA. Craniofacial ciliopathies: A new classification for craniofacial disorders. *Am J Med Genet A* 2010; 152A:2995-3006; PMID:21108387; <http://dx.doi.org/10.1002/ajmg.a.33727>
- Macca M, Franco B. The molecular basis of oral-facial-digital syndrome, type 1. *Am J Med Genet C Semin Med Genet* 2009; 151C:318-25; PMID:19876934; <http://dx.doi.org/10.1002/ajmg.c.30224>
- Walczak-Sztulpa J, Eggenchwiler J, Osborn D, Brown DA, Emma F, Klingenberg C, Hennekam RC, Torre G, Garshasbi M, Tzschach A, et al. Cranioectodermal Dysplasia, Sensenbrenner syndrome, is a ciliopathy caused by mutations in the IFT122 gene. *Am J Hum Genet* 2010; 86:949-56; PMID:20493458; <http://dx.doi.org/10.1016/j.ajhg.2010.04.012>
- Levin LS, Perrin JC, Ose L, Dorst JP, Miller JD, McKusick VA. A heritable syndrome of craniosynostosis, short thin hair, dental abnormalities, and short limbs: cranioectodermal dysplasia. *J Pediatr* 1977; 90:55-61; PMID:830894; [http://dx.doi.org/10.1016/S0022-3476\(77\)80764-6](http://dx.doi.org/10.1016/S0022-3476(77)80764-6)
- Seo JH, Zilber Y, Babayeva S, Liu J, Kyriakopoulos P, De Marco P, Merello E, Capra V, Gros P, Torban E. Mutations in the planar cell polarity gene, *Fuzzy*, are associated with neural tube defects in humans. *Hum Mol Genet* 2011; 20:4324-33; PMID:21840926; <http://dx.doi.org/10.1093/hmg/ddr359>
- Park TJ, Haigo SL, Wallingford JB. Ciliogenesis defects in embryos lacking inturned or fuzzy function are associated with failure of planar cell polarity and Hedgehog signaling. *Nat Genet* 2006; 38:303-11; PMID:16493421; <http://dx.doi.org/10.1038/ng1753>
- Heydeck W, Zeng H, Liu A. Planar cell polarity effector gene *Fuzzy* regulates cilia formation and Hedgehog signal transduction in mouse. *Dev Dyn* 2009; 238:3035-42; PMID:19877275; <http://dx.doi.org/10.1002/dvdy.22130>
- Gray RS, Abitua PB, Wlodarczyk BJ, Szabo-Rogers HL, Blanchard O, Lee I, Weiss GS, Liu KJ, Marcotte EM, Wallingford JB, et al. The planar cell polarity effector *Fuz* is essential for targeted membrane trafficking, ciliogenesis and mouse embryonic development. *Nat Cell Biol* 2009; 11:1225-32; PMID:19767740; <http://dx.doi.org/10.1038/ncb1966>
- Tabler JM, Barrell WB, Szabo-Rogers HL, Healy C, Yeung Y, Perdiguer EG, Schulz C, Yannakoudakis BZ, Mesbah A, Wlodarczyk B, et al. Fuz mutant mice reveal shared mechanisms between ciliopathies and FGF-related syndromes. *Dev Cell* 2013; 25:623-35; PMID:23806618; <http://dx.doi.org/10.1016/j.devcel.2013.05.021>
- Cohen MM Jr., Kreiborg S. Skeletal abnormalities in the Apert syndrome. *Am J Med Genet* 1993; 47:624-32; PMID:8266987; <http://dx.doi.org/10.1002/ajmg.1320470509>
- Hennekam R, et al. Gorlin's Syndromes of the Head and Neck Oxford University Press, USA, 2010.
- Hemmer KM, McAlister WH, Marsh JL. Cervical spine anomalies in the craniosynostosis syndromes. *Cleft Palate J* 1987; 24:328-33; PMID:3479279
- Moore MH, Cantrell SB, Trott JA, David DJ. Pfeiffer syndrome: a clinical review. *Cleft Palate Craniofac J* 1995; 32:62-70; PMID:7772489; [http://dx.doi.org/10.1597/1545-1569\(1995\)032<0062:PSACR>2.3.CO;2](http://dx.doi.org/10.1597/1545-1569(1995)032<0062:PSACR>2.3.CO;2)
- Moore MH, Lodge ML, Clark BE. Spinal anomalies in Pfeiffer syndrome. *Cleft Palate Craniofac J* 1995; 32:251-4; PMID:7605793; [http://dx.doi.org/10.1597/1545-1569\(1995\)032<0251:SAIPS>2.3.CO;2](http://dx.doi.org/10.1597/1545-1569(1995)032<0251:SAIPS>2.3.CO;2)
- Anderson PJ, Hall CM, Evans RD, Hayward RD, Harkness WJ, Jones BM. The cervical spine in Saethre-Chotzen syndrome. *Cleft Palate Craniofac J* 1997; 34:79-82; PMID:9003917; [http://dx.doi.org/10.1597/1545-1569\(1997\)034<0079:TCSISC>2.3.CO;2](http://dx.doi.org/10.1597/1545-1569(1997)034<0079:TCSISC>2.3.CO;2)
- Khanna AJ, Braverman NE, Valle D, Sponseller PD. Cervical stenosis secondary to rhizomelic chondrodysplasia punctata. *Am J Med Genet* 2001; 99:63-6; PMID:11170096; [http://dx.doi.org/10.1002/1096-8628\(20010215\)99:1<63::AID-AJMG1117>3.0.CO;2-9](http://dx.doi.org/10.1002/1096-8628(20010215)99:1<63::AID-AJMG1117>3.0.CO;2-9)
- Sparrow DB, Chapman G, Smith AJ, Mattar MZ, Major JA, O'Reilly VC, Saga Y, Zackai EH, Dormans JP, Alman BA, et al. A mechanism for gene-environment interaction in the etiology of congenital scoliosis. *Cell* 2012; 149:295-306; PMID:22484060; <http://dx.doi.org/10.1016/j.cell.2012.02.054>
- Hajihosseini MK, Lalioti MD, Arthaud S, Bugar HR, Brown JM, Twigg SR, Wilkie AO, Heath JK. Skeletal development is regulated by fibroblast growth factor receptor 1 signalling dynamics. *Development* 2004; 131:325-35; PMID:14668415; <http://dx.doi.org/10.1242/dev.00940>
- Juric-Sekhar G, Adkins J, Doherty D, Hevner RF. Joubert syndrome: brain and spinal cord malformations in genotyped cases and implications for neurodevelopmental functions of primary cilia. *Acta Neuropathol* 2012; 123:695-709; PMID:22331178; <http://dx.doi.org/10.1007/s00401-012-0951-2>
- Hajihosseini MK, Wilson S, De Moerloose L, Dickson C. A splicing switch and gain-of-function mutation in *Fgfr2-IIIc* hemizygotes causes Apert/Pfeiffer-syndrome-like phenotypes. *Proc Natl Acad Sci U S A* 2001; 98:3855-60; PMID:11274405; <http://dx.doi.org/10.1073/pnas.071586898>
- Wang Y, Xiao R, Yang F, Karim BO, Iacovelli AJ, Cai J, Lerner CP, Richtsmeier JT, Leszl JM, Hill CA, et al. Abnormalities in cartilage and bone development in the Apert syndrome *FGFR2(+S252W)* mouse. *Development* 2005; 132:3537-48; PMID:15975938; <http://dx.doi.org/10.1242/dev.01914>
- Wang Q, Green RP, Zhao G, Ornitz DM. Differential regulation of endochondral bone growth and joint development by *FGFR1* and *FGFR3* tyrosine kinase domains. *Development* 2001; 128:3867-76; PMID:11585811
- Hoeffel JC, Pernot C, Juncker P. Radiologic patterns of the sternum in Noonan's syndrome with congenital heart defect. *Am J Dis Child* 1981; 135:1044-6; PMID:6794358
- Tartaglia M, Kalidas K, Shaw A, Song X, Musat DL, van der Burgt I, Brunner HG, Bertola DR, Crosby A, Ion A, et al. PTPN11 mutations in Noonan syndrome: molecular spectrum, genotype-phenotype correlation, and phenotypic heterogeneity. *Am J Hum Genet* 2002; 70:1555-63; PMID:11992261; <http://dx.doi.org/10.1086/340847>
- Zhang Q, Murcia NS, Chittenden LR, Richards WG, Michaud EJ, Woychik RP, Yoder BK. Loss of the Tg737 protein results in skeletal patterning defects. *Dev Dyn* 2003; 227:78-90; PMID:12701101; <http://dx.doi.org/10.1002/dvdy.10289>
- Cui C, Chatterjee B, Francis D, Yu Q, SanAgustin JT, Francis R, Tansey T, Henry C, Wang B, Lemley B, et al. Disruption of *Mks1* localization to the mother centriole causes cilia defects and developmental malformations in Meckel-Gruber syndrome. *Dis Model Mech* 2011; 4:43-56; PMID:21045211; <http://dx.doi.org/10.1242/dmm.006262>
- Mo R, Freer AM, Zinyk DL, Crackower MA, Michaud J, Heng HH, Chik KW, Shi XM, Tsui LC, Cheng SH, et al. Specific and redundant functions of *Gli2* and *Gli3* zinc finger genes in skeletal patterning and development. *Development* 1997; 124:113-23; PMID:9006072
- Waters A, et al. Bardet-Biedl Syndrome. In: RA P, et al., eds. *GeneReviews*. Seattle (WA): University of Washington, Seattle, 2011.
- Ho NC, Francomano CA, van Allen M. Jeune asphyxiating thoracic dystrophy and short-rib polydactyly type III (Verma-Naumoff) are variants of the same disorder. *Am J Med Genet* 2000; 90:310-4; PMID:10710229; [http://dx.doi.org/10.1002/\(SICI\)1096-8628\(20000214\)90:4<310::AID-AJMG9>3.0.CO;2-N](http://dx.doi.org/10.1002/(SICI)1096-8628(20000214)90:4<310::AID-AJMG9>3.0.CO;2-N)
- Bredrup C, Saunier S, Oud MM, Fiskerstrand T, Hoischen A, Brackman D, Leh SM, Midtbø M, Filhol E, Bole-Feyso C, et al. Ciliopathies with skeletal anomalies and renal insufficiency due to mutations in the IFT-A gene *WDR19*. *Am J Hum Genet* 2011; 89:634-43; PMID:22019273; <http://dx.doi.org/10.1016/j.ajhg.2011.10.001>
- Sensenbrenner JA, Dorst JP, Owens RP. New syndrome of skeletal, dental and hair anomalies. *Birth Defects Orig Artic Ser* 1975; 11:372-9; PMID:1227553
- Young ID. Cranioectodermal dysplasia (Sensenbrenner's syndrome). *J Med Genet* 1989; 26:393-6; PMID:2661822; <http://dx.doi.org/10.1136/jmg.26.6.393>
- Smith HL, Hand AM. Chondroectodermal dysplasia (Ellis-van Creveld syndrome); report of two cases. *Pediatrics* 1958; 21:298-307; PMID:13505024

43. Weatherbee SD, Niswander LA, Anderson KV. A mouse model for Meckel syndrome reveals Mks1 is required for ciliogenesis and Hedgehog signaling. *Hum Mol Genet* 2009; 18:4565-75; PMID:19776033; <http://dx.doi.org/10.1093/hmg/ddp422>
44. Pacheco M, Valencia M, Caparrós-Martín JA, Mulero F, Goodship JA, Ruiz-Perez VL. Evc works in chondrocytes and osteoblasts to regulate multiple aspects of growth plate development in the appendicular skeleton and cranial base. *Bone* 2012; 50:28-41; PMID:21911092; <http://dx.doi.org/10.1016/j.bone.2011.08.025>
45. Ruiz-Perez VL, Blair HJ, Rodríguez-Andrés ME, Blanco MJ, Wilson A, Liu YN, Miles C, Peters H, Goodship JA. Evc is a positive mediator of Ihh-regulated bone growth that localises at the base of chondrocyte cilia. *Development* 2007; 134:2903-12; PMID:17660199; <http://dx.doi.org/10.1242/dev.007542>
46. Ferrante MI, Zullo A, Barra A, Bimonte S, Messaddeq N, Studer M, Dollé P, Franco B. Oral-facial-digital type I protein is required for primary cilia formation and left-right axis specification. *Nat Genet* 2006; 38:112-7; PMID:16311594; <http://dx.doi.org/10.1038/ng1684>
47. Bimonte S, De Angelis A, Quagliata L, Giusti F, Tammaro R, Dallai R, Ascenzi MG, Diez-Roux G, Franco B. Odf1 is required in limb bud patterning and endochondral bone development. *Dev Biol* 2011; 349:179-91; PMID:20920500; <http://dx.doi.org/10.1016/j.ydbio.2010.09.020>
48. Mill P, Lockhart PJ, Fitzpatrick E, Mountford HS, Hall EA, Reijns MA, Keighren M, Bahlo M, Bromhead CJ, Budd P, et al. Human and mouse mutations in WDR35 cause short-rib polydactyly syndromes due to abnormal ciliogenesis. *Am J Hum Genet* 2011; 88:508-15; PMID:21473986; <http://dx.doi.org/10.1016/j.ajhg.2011.03.015>
49. Rynearson RD. Case report: orthodontic and dentofacial orthopedic considerations in Apert's syndrome. *Angle Orthod* 2000; 70:247-52; PMID:10926435
50. Anderson PJ, Hall C, Evans RD, Harkness WJ, Hayward RD, Jones BM. The cervical spine in Crouzon syndrome. *Spine (Phila Pa 1976)* 1997; 22:402-5; PMID:9055367; <http://dx.doi.org/10.1097/00007632-199702150-00009>
51. Eswarakumar VP, Ozcan F, Lew ED, Bae JH, Tomé F, Booth CJ, Adams DJ, Lax I, Schlessinger J. Attenuation of signaling pathways stimulated by pathologically activated FGF-receptor 2 mutants prevents craniosynostosis. *Proc Natl Acad Sci U S A* 2006; 103:18603-8; PMID:17132737; <http://dx.doi.org/10.1073/pnas.0609157103>
52. Perlyn CA, DeLeon VB, Babbs C, Govier D, Burell L, Darvann T, Kreiborg S, Morriss-Kay G. The craniofacial phenotype of the Crouzon mouse: analysis of a model for syndromic craniosynostosis using three-dimensional MicroCT. *Cleft Palate Craniofac J* 2006; 43:740-8; PMID:17105336; <http://dx.doi.org/10.1597/05-212>
53. Bellus GA, McIntosh I, Smith EA, Aylsworth AS, Kaitila I, Horton WA, Greenhaw GA, Hecht JT, Francomano CA. A recurrent mutation in the tyrosine kinase domain of fibroblast growth factor receptor 3 causes hypochondroplasia. *Nat Genet* 1995; 10:357-9; PMID:7670477; <http://dx.doi.org/10.1038/ng0795-357>
54. Elejalde BR, de Elejalde MM. Thanatophoric dysplasia: fetal manifestations and prenatal diagnosis. *Am J Med Genet* 1985; 22:669-83; PMID:4073120; <http://dx.doi.org/10.1002/ajmg.1320220404>
55. Georgoulis G, Alexiou G, Prodromou N. Achondroplasia with synostosis of multiple sutures. *Am J Med Genet A* 2011; 155A:1969-71; PMID:21739570; <http://dx.doi.org/10.1002/ajmg.a.33744>
56. Murakami S, Balmes G, McKinney S, Zhang Z, Givol D, de Crombrughe B. Constitutive activation of MEK1 in chondrocytes causes Stat1-independent achondroplasia-like dwarfism and rescues the Fgfr3-deficient mouse phenotype. *Genes Dev* 2004; 18:290-305; PMID:14871928; <http://dx.doi.org/10.1101/gad.1179104>
57. Chen L, Adar R, Yang X, Monsonego EO, Li C, Hauschka PV, Yayon A, Deng CX. Gly369Cys mutation in mouse FGFR3 causes achondroplasia by affecting both chondrogenesis and osteogenesis. *J Clin Invest* 1999; 104:1517-25; PMID:10587515; <http://dx.doi.org/10.1172/JCI6690>